Open Access

Original Article

Effects of Estrogen on Histomorphology Steroid-Induced Avascular Necrosis of Head of Femur in Male Rats

Sadaf Iqbal, Shabnam Hamid, Faiza Umbreen, Mahwash Azam, Ayesha Ali, Maroosh Mirza

Department of Anatomy, Army Medical College/National University of Medical Sciences, (NUMS) Rawalpindi Pakistan

ABSTRACT

Objectives: To study the effects of estrogen administration on histomorphology of avascular necrosis of the femoral head in male rats.

Study Design: Lab-based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, National University of Medical Sciences, Rawalpindi (NUMS), in collaboration with the National Institute of Health Islamabad, (NIH) Pakistan from Aug to Nov 2021.

Methodology: Thirty male Sprague Dawley rats, three months of age, weighing 200-300gm, were selected. Rats were equally divided into three groups. Group-A served as a control group in which no intervention was made, and the rats were fed on a standard lab diet. Groups B and C served as experimental groups. In Groups B and C, avascular necrosis was induced by steroids for the first week of the study. Group C along with steroids also received tab estrogen by oral gavage for eight weeks, starting from the fifth week of the study till the twelfth week of the study. All the rats were sacrificed after the completion of the experimental period. The microscopic parameters like cortical thickness and percentage of the empty lacunae were counted.

Results: Histomorphological changes were observed when the comparison was made between Group B, Control Group A, and Experimental Groups B and C with statistically significant results (*p*-value 0.001).

Conclusion: Estrogen helps in fracture healing and shows improvement in cortical and trabecular strength. It has shown improvement in the cortical thickness measurement after avascular necrosis causes a decrease in the thickness.

Keywords: Avascular necrosis, Estrogen, Head of the femur, Steroids.

How to Cite This Article: *Iqbal S, Hamid S, Umbreen F, Azam M, Ali A, Mirza M. Effects of Estrogen on Histomorphology Steroid-Induced Avascular Necrosis of Head of Femur in Male Rats. Pak Armed Forces Med J 2023; 73(6): 1733-1736. DOI: https://doi.org/10.51253/pafmj.v73i6.8769*

INTRODUCTION

Avascular necrosis, AVN or osteonecrosis, is a disease of the bones. It occurs as a result of the impaired blood supply to the bone.[1,2] This insult to the bone due to decreased blood supply results in the death of bone-forming cells like osteoblasts and osteocytes. The number of osteoclasts increases, consequently increasing bone resorption rather than new bone formation. The impaired bone formation ultimately results in the collapse of the bone.[3,4] Avascular necrosis involves a series of mechanisms, which include mechanical disruption, Intravascular occlusion, and extravascular compression. There is impaired lipid metabolism, hypercoagulable state, thrombus formation and ultimately, ischemia, causing a decreased blood supply to the bone.[5,6]

Estrogen is a sex hormone that develops the reproductive system and secondary sexual characteristics in females. Although predominately a female hormone, its role in males has also been established in the studies.[7] This is documented by estrogen receptors in the male genital system. The role of estrogen has also been documented in the homeostasis of the bone. In postmenopausal women, estrogen deficiency results in the increased chances of osteoporosis.[8]

Estrogen helps maintain the bone skeleton by decreasing bone resorption and increasing remodelling. The effects of estrogen on avascular necrosis have not been studied before.[9] Considering these facts, the study was designed to perceive the ameliorative effects of estrogen administration on the avascular necrosis of bones. The patients should be followed up frequently to evaluate any undesirable effects on the body.[10] This study was conducted to observe the effects of estrogen administration on histomorphology of avascular necrosis of the femoral head in male rats.

METHODOLOGY

The laboratory-based experimental study was conducted at the Department of Anatomy, Army Medical College, National University of Medical Sciences, Rawalpindi, in collaboration with the National Institute of Health (NIH) Islamabad, Pakistan from August to November 2021, following approval

Correspondence: Dr Sadaf Iqbal, Department of Anatomy, Army Medical College, Rawalpindi Pakistan

Received:23 May 2022;revision received: 01 May 2023; accepted: 03 May 2023

from the Ethics Review Committee of Army Medical College, National University of Medical Sciences (ERC/ID/127). The sampling technique used was non-probability convenience sampling

Inclusion Criteria: Male Sprague Dawley rats of 3-4 months having an average weight of 250±50g were included.

Exclusion Criteria: Rats with any obvious bone deformity were excluded.

Thirty male Sprague Dawley rats, 3 to 4 months of age, weighing 250±50 grams, were procured from NIH, Islamabad. The animals were divided into three equal groups. Each group comprised ten rats, and each group was kept in a separate cage in the animal house of NIH under standard laboratory conditions with an optimal temperature of 21±2C and a 12-hour light/ dark cycle. The animals were fed on standard laboratory rat chow and water ad libitum. Rats in Group A served as a Control Group in which no intervention was made. Rats in Groups B and C received Inj Methylprednisolone 40mg/kg/body weight intraperitoneally for three consecutive days to induce avascular necrosis in femoral heads. Rats in Group C and steroids received tab estrogen at 0.1mg/kg body weight daily by oral gavage for the eighth week, starting from the fifth week of the study period till the twelfth week.

The cortical bone thickness and percentage of empty lacunae were calculated microscopically in the femoral head of the rats (Figure-1 & 2). The cortical bone thickness was determined by drawing a perpendicular line from the periosteum to the endosteum.[11,12] The cortical thickness was measured by using micrometry. Three consecutive high-power fields were selected for each specimen. Three areas per high field were studied, and the mean was calculated.

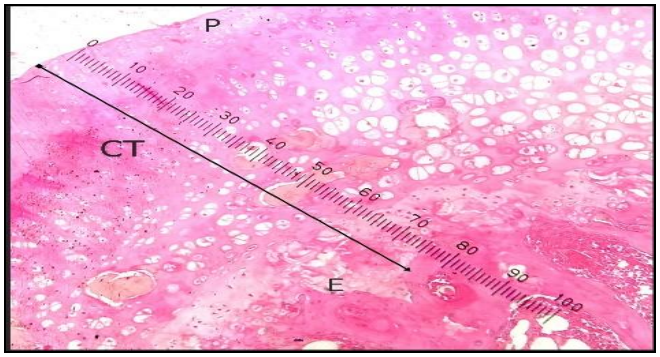

Figure-1: Photomicrograph of histological section of head of femur showing measurement of cortical thickness(CT) from periosteum(P)to endosteum(E). H & E 400X

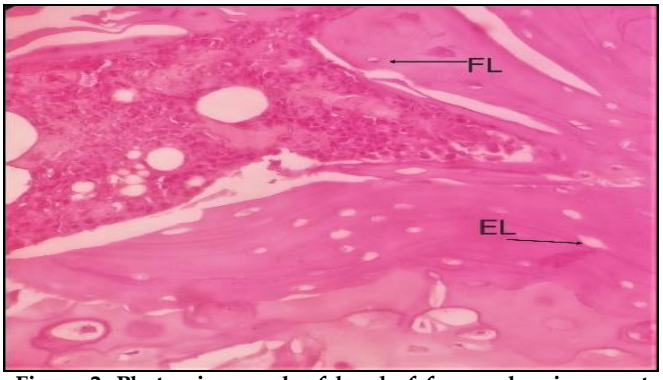

Figure-2: Photomicrograph of head of femur showing empty (EL) and filled lacunae (FL). H & E 400X

The percentage of empty lacunae was counted in the whole slide. The total number of lacunae included empty as well as filled lacunae.[13] They were counted at HPF. The calculation was made on screen by an Olympus digital microscope connected with LED.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. One-way analysis of variance (ANOVA) was applied to gauge the mean differences among the groups. The group differences were calculated using Post Hoc test (Tukey HSD). The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

Thirty male Sprague Dawley rats, were selected. Rats were equally divided into three groups. The mean cortical thickness in Group-A was 22.20µm±1.77. The Experimental Groups B and C showed the cortical thickness of 18.77µm±1.73 and 21.45µm±1.35, respectively. The intergroup comparison revealed a significant result, a *p*-value of 0.001. The intergroup comparison between Groups A and B and Groups B and C showed significant results with p-values of 0.001 and *p*-values of 0.003, respectively. At the same time, Groups A and C revealed insignificant values of 0.561 (Table-I).

The mean percentage of the empty lacunae calculated in control Group-A was 3.14 ±0.75. The percentage of empty lacunae in the Experimental Groups B and C were calculated as 10.35 ± 0.86 and 4.76 ±0.59, respectively. The Group comparison was significant, with a p-value of 0.001. The intergroup comparison between A and B, B and C and between A and C revealed significant results with a *p*-value of 0.001 (Table-II).

Table-I: Mean Cortical thickness and Percentage of empty lacunae in Control Group A and Experimental Groups B and C (n=30)

Parameters	Group-A Control Means±SD (n=10)	Group-B Experimental Means±SD (n=10)	Group-C Experimental Means±SD (n=10)	*p*-value
Cortical thickness (µm)	22.20±1.77	18.77±1.73	21.45±1.35	0.001**
Percentage of Empty Lacunae	3.41±0.76	10.35±0.086	4.76±0.59	0.001**

Table-II: Post Hoc Analysis Cortical thickness and Percentage of Empty Lacunae in Control Group A and Experimental Group B and C (n=30)

Parameters	Group-A vs Group-B	Group-A vs Group- C	Group-B vs Group-C
Cortical thickness (µm)	0.001**	0.561	0.003*
Percentage of empty lacunae	0.001**	0.001**	0.001**

DISCUSSION

The role of estrogen on bones has been established in the studies. Improvement in bone mineral density and fracture healing in postmenopausal women after treatment with estrogen has been documented.[11,12] Estrogen also plays an important role in maintaining the bone health of males as well. Research has shown that in males, rapid bone loss occurs after castration. Estrogen deficiency causes loss of cancellous bone and narrowing in the cortical bone width.[13] The amount of bone resorption outnumbers the new bone formation. The impact of the estrogen on the bone is mediated through estrogen receptors, i.e. ERα and ERβ.[14,15] Even though estrogen is a sex hormone, its receptors have been detected in bone cells. Estrogen plays its role at the molecular level through signalling pathways. Bone loss will occur if the receptors are deleted, or estrogen is insufficient.[16] In our study, avascular necrosis was induced in Groups B and C rats using Inj. Methylprednisolone. Once the avascular necrosis had been established, rats in Group C were given a tablet of estrogen, which was given by oral gavage to see the ameliorative effects of estrogen on bones. The cortical bone thickness was found to be reduced once the avascular necrosis had been established. This was shown when the control was compared with experimental group B, and the results were found to be significant, with a p-value of 0.001. This was found to be consistent with the studies held previously.[17,18] When Group C was treated with estrogen, the femoral heads showed improvement in cortical bone thickness. The statistically significant results with a *p*-value of 0.003 were found when the intergroup comparison between groups B and C was made. This was relevant to the previously conducted study.[19]

The percentage of empty lacunae was increased once the femoral head was induced with avascular necrosis. It was calculated in the whole slide. The comparison among the groups revealed significant results with a *p*-value of 0.001. The increased percentage of the empty lacunae was seen in group B compared to group A. This was in accordance with the study held by Fan *et al*.[20]

The role of estrogen on cortical bone thickness is due to its effects on the osteoblasts. Recent studies showed that estrogen receptors ERα promote the signalling pathway for maintaining cortical and cancellous bone health. These receptors are present in the osteoblasts. Thus, osteoblasts are indirectly responsible for the bone microstructure.

ACKNOWLEDGEMENT

The authors were highly obliged to the National University of Medical Science (NUMS), Rawalpindi, to finance our study.

CONCLUSION

The role of estrogen on the skeleton has been documented in the studies. It helps in fracture healing and shows improvement in cortical and trabecular strength. This was shown by the improvement in the cortical thickness measurement after avascular necrosis causes a decrease in the thickness. In addition, there was a decrease in the percentage of empty lacunae when the rats were treated with estrogen. Thus, it can be concluded that estrogen has ameliorative effects on AVN, with special emphasis on the duration and dosage of estrogen required for the treatment of AVN.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SI & SH: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

FU & MA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

AA & MM: Data acquisition, data analysis, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES:

1. Wei J-H, Luo Q-Q, Tang Y-J, Chen J-X, Huang C-L, Lu D-G, et al. Upregulation of microRNA-320 decreases the risk of developing steroid-induced avascular necrosis of femoral head by inhibiting CYP1A2 both in vivo and in vitro. Gene 2018: 660(1): 136-144. https://doi.org/10.1016/j.gene.2018.03.045
2. Cheng C-H, Chen L-R, Chen K-H. Osteoporosis Due to Hormone Imbalance: An Overview of the Effects of Estrogen Deficiency and Glucocorticoid Overuse on Bone Turnover. Int J Mol Sci 2022; 23(3): 1376. https://doi.org/10.3390/ijms23031376
3. Shaikh SA, Hussain S, Samejo MQA, Ahmed N, Jamali AR. Osetosynthesis of Fractures neck femur with cannulated screws: Evaluation of risk factors for post-operative complications. J Pak Med Assoc 2021; 71(Suppl-5): S59-S63.
4. Petek D, Hannouche D, Suva D. Osteonecrosis of the femoral head: pathophysiology and current concepts of treatment. EFORT Open Rev 2019; 4(3): 85-97. https://doi.org/10.1302/2058-5241.4.180036
5. Xu J, Gong H, Lu S, Deasey MJ, Cui QJ. Animal models of steroid-induced osteonecrosis of the femoral head-a comprehensive research review up to 2018. Int Orthop 2018 ; 42(7): 1729-1737. https://doi.org/10.1007/s00264-018-3956-1
6. Baig SA, Baig M. Osteonecrosis of the femoral head: etiology, investigations, and management. Cureus 2018; 10(8): 1-4. https://doi.org/10.7759/cureus.3171
7. Ye T, Cao P, Qi J, Zhou Q, Rao DS, Qiu SJ. Protective effect of low-dose risedronate against osteocyte apoptosis and bone loss in ovariectomized rats. PLoS One 2017; 12(10): e0186012. https://doi.org/10.1371/journal.pone.0186012
8. Broulik PD, Urbánek V, Libanský PJH, Research M. Eighteen-year effect of androgen therapy on bone mineral density in trans (gender) men. Horm Metab Res 2018; 50(02): 133-137. https://doi.org/10.1055/s-0043-118747
9. Cooke PS, Nanjappa MK, Ko C, Prins GS, Hess RA. Estrogens in male physiology. Physiol Rev 2017; 97(3): 995-1043. https://doi.org/10.1152/physrev.00018.2016
10. Cui J, Shen Y, Li RJ. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med 2013; 19(3): 197–209. https://doi.org/10.1016/j.molmed.2012.12.007
11. Coates BA, McKenzie JA, Yoneda S, Silva MJ. Interleukin-6 (IL-6) deficiency enhances intramembranous osteogenesis following stress fracture in mice. Bone 2021; 143(1): 115737. https://doi.org/10.1016%2Fj.bone.2020.115737
12. Surve VV, Andersson N, Lehto-Axtelius D, Håkanson R. Comparison of osteopenia after gastrectomy, ovariectomy and prednisolone treatment in the young female rat. Acta Orthop Scand 2001; 72(5): 525-532. https://doi.org/10.1080/000164701753532880
13. Aghaloo TL, Kang B, Sung EC, Shoff M, Ronconi M, Gotcher JE, et al. Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. J Bone Miner Res 2011; 26(8): 1871-1882. https://doi.org/10.1002/jbmr.379
14. Tahami M, Haddad B, Abtahian A, Hashemi A, Aminian A, Konan S,et al. Potential role of local estrogen in enhancement of fracture healing: preclinical study in rabbits. Arch Bone Joint Surg 2016; 4(4): 323-326.
15. Khosla S, Monroe DG. Regulation of bone metabolism by sex steroids. Cold Spring Harb Perspect Med 2018 ; 8(1): a031211. https://doi.org/10.1101%2Fcshperspect.a031211
16. Tamer SA, Yıldırım A, Arabacı Ş, Çiftçi S, Akın S, Sarı E, et al. Treatment with estrogen receptor agonist ERβ improves torsion-induced oxidative testis injury in rats. Life Sci 2019 : 222: 203-211. https://doi.org/10.1016/j.lfs.2019.02.056
17. Tobeiha M, Moghadasian MH, Amin N, Jafarnejad SJ. RANKL/RANK/OPG pathway: a mechanism involved in exercise-induced bone remodeling. Biomed Res Int 2020; 2020: 6910312. https://doi.org/10.1155%2F2020%2F6910312
18. Farman HH, Gustafsson KL, Henning P, Grahnemo L, Lionikaite V, Movérare-Skrtic S, et al. Membrane estrogen receptor α is essential for estrogen signaling in the male skeleton. J Endocrinol 2018; 239(3): 303-312. https://doi.org/10.1530/joe-18-0406
19. Mont MA, Salem HS, Piuzzi NS, Goodman SB, Jones LC. Nontraumatic osteonecrosis of the femoral head: where do we stand today?: a 5-year update. J Bone Joint Surg Am 2020 ; 102(12): 1084-1099. https://doi.org/10.2106/jbjs.19.01271
20. Fan ZQ, Bai SC, Xu Q, Li ZJ, Cui WH, Li H, et al. Oxidative Stress Induced Osteocyte Apoptosis in Steroid-Induced Femoral Head Necrosis. Orthop Surg 2021; 13(7): 2145–2152. https://doi.org/10.1111%2Fos.13127